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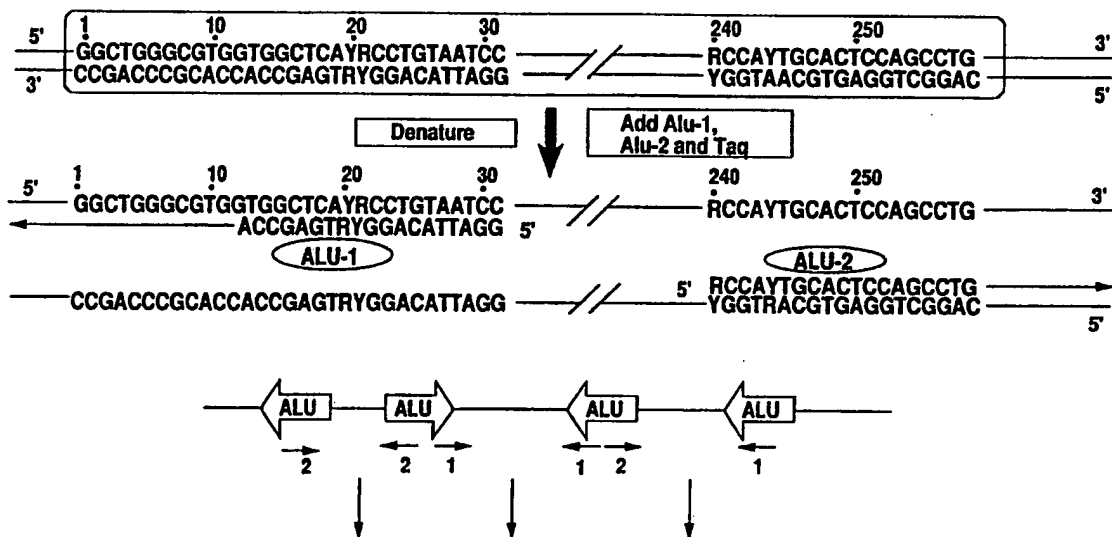
(57) Abstract

The invention relates to novel primer sets useful in preparing DNA probes specific for any chromosome or part of a chromosome, particularly human chromosomes. The DNA probes so produced may be used to paint individual chromosomes or portions of chromosomes in metaphase cell spreads and in interphase nuclei. When used to paint chromosomes in metaphase spreads, R-bands are readily detectable. The method is sensitive and has been shown to paint R-bands on chromosomes pieces having as few as several hundred kilobases.

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+ DESIGNATIONS OF "SU"

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IN-SITU HYBRIDIZATION PROBES FOR IDENTIFICATION AND BANDING OF SPECIFIC HUMAN CHROMOSOMES AND REGIONS

5 The invention relates generally to the preparation of chromosome specific DNA probes that are useful in selectively detecting individual chromosomes or chromosome parts. The method may be used for detection of chromosomes or parts of chromosomes in metaphase cell spreads and in interphase nuclei by *in-situ*
10 hybridization.

Chromosome identification procedures have long been considered an integral aspect of biomedical diagnostic practice because of the many disease syndromes having a
15 genetic basis which are caused or diagnosed by chromosomal alterations. Being able to observe these chromosomal alterations in cells of the body, therefore, not only helps in the diagnosis of the disease but has the potential of being an effective monitor of
20 therapeutic procedures designed to eliminate the cells with the chromosomal anomalies from the body. It is also important to be able to rapidly monitor the chromosomes of the fetuses of pregnancies at risk (such as in older women, or where a parent may have been exposed to
25 environmental mutagen, or where previous siblings of the fetus have an abnormality with known chromosomal basis). Specific chromosomal abnormalities -- translocations (exchanges between the arms of two different chromosomes), inversions (internal segment of a
30 chromosome swings around and faces the opposite direction), deletions (piece of chromosome becomes lost), monosomies (only one instead of two chromosomes of a type), and trisomies (three instead of two chromosomes of a type) -- have been associated with specific sub-types
35 of cancer (1) as well as in a wide variety of inherited r congenital abnormalities (2).

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Chromosomally based disease syndromes are often seen
in hematological malignancies such as leukemia and
lymphoma. The classic example is the 9:22 translocation
associated with chronic myelogenous leukemia (CML).
5   Here, there is a reciprocal translocation between the end
of the long arm of chromosome 9 and the middle of the
long arm of chromosome 22 producing what is known as the
Philadelphia (Ph) chromosome of the translocation
chromosome carrying the chromosome 22 centromere (3-5).
10   The ability to see such events was made possible by a
staining method developed in the early '70s which
imparted to each of the 24 different human chromosomes a
distinctive banding pattern (6,7). Therefore, by
carrying out such a stain on cells and photographing
15   them, individual chromosomes can be cut out and lined up
(according to their bands) in a procedure called
karyotyping. By doing this the translocation, or any
abnormal chromosome eventually stands out from the rest
and is identified.
20

While effective and important in original diagnosis,
this procedure is impractical for determining the
percentage of such cancer cells in patients with minimal
residual disease or in relapse because it is arduous and
25   labor-intensive. Furthermore, with respect to monitoring
pregnancies, a faster method is preferred. A
disadvantage of the procedure is its dependence on high
quality metaphase spreads (structures formed when the
chromosomes condense and form their distinctive
30   morphology and banding pattern during the division phase
of the cell cycle). Good, scorable metaphases are often
not available in the tissues which one needs to monitor
(e.g., bone marrow which is the source of many leukemic
cells, or amniocentesis tissues for fetus evaluation).
35   The result is that rearranged chromosomes are often not
identifiable by this method leaving the entities f

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unknown origin to be referred to as "marker chromosomes".

Two groups (8,9) have described a method to stain a
specific chromosome of choice. The problem associated
5   with such a procedure was to obtain DNA from only the
chromosome of interest, label it in some way so that it
would be identified later (this labeled DNA is therefore
chromosome-specific "probe"), and then hybridize probe to
the chromosomes in a cell on a microscope slide. By
10   visualizing the label, the chromosome was visualized
since the DNA, if properly handled, hybridized only to
the chromosome from which it was derived. The procedure
has since come to be known as chromosome painting. Using
a human chromosome 21 probe, trisomies and translocations
15   associated with the chromosome (9,10) were visualized
even inn poorly d efined metaphases. Specific
chromosomes of interest used to prepare painting probes
have been separated from other chromosomes by flow-
sorting synchronized populations of dividing cells
20   through a technically rigorous procedure requiring highly
specialized and expensive equipment (a fluorescence-
activated cell sorter). This leads to only a small
quantity of material, so that the DNAs from the
individual chromosomes then need to be extracted and
25   cloned into cosmid libraries. To make probe, the cosmid
library must be expanded, DNA extracted again and nick-
translated in the presence of biotinylated nucleotide
prior to hybridization to human metaphase spreads and
detection of the specific chromosomes (11). An essential
30   requirement for specific chromosome painting is the
prehybridization of the probe with total human DNA in
order to prevent human repeat sequences (which are not
chromosome specific) from participating in the *in-situ*

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hybridization reaction.

Unfortunately, flow sorting of individual
chromosomes, making and expanding and maintaining cosmid
5   libraries is problematical because of possible
contamination with other chromosomes or the presence of
non-chromosomal specific sequences in such material that
cannot be prevented from participating in the *in-situ*
hybridization. These concerns have been recently
10   highlighted (12) in studies where such probe made for
human chromosome 22 hybridized to additional chromosomal
regions on human metaphases. Other serious limitations
of the approach include: 1) cross-hybridization of the
chromosome 2 probe to the centromere of chromosome 19; 2)
15   failure of libraries made from chromosome 5 to paint
chromosome 5; 3) cross-hybridization of probes made from
flow-sorted chromosomes 13, 14, 15, 21, and 22 to the
centromeric regions of each other; and 4) cross-
hybridization of the chromosome 18 probe to the
20   centromeric regions of chromosomes 12 and 19 (as in
poster presentation at the 41st Annual Meeting of the
American society of Human Genetics -- 13). The method is
also expensive and limited to the availability of the
flow sorted libraries. The procedure is relatively
25   inflexible. For instance, there are situations in which
one might be interested in painting only a portion of a
specific chromosome (e.g., the p-arm of chromosome 16).
This has not yet been achieved using flow-sorted
libraries, yet this would be an excellent probe for the
30   identification of inversions associated with cancer and
other disease syndromes. An example of this is acute
nonlymphocytic leukemia where there is a pericentric
inversion involving both the short and long arms of
chromosome 16 (14). Probe f r only the short arm of th
35   chromosome would identify the inversion chromosome as one
which had staining on portions of both arms whereas a

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normal chromos me 16 would have only the short arm
painted. Finally, probe made from flow sorted chromosome
libraries does not allow the identification of the
regions of the respective chromosomes brought together by
5   the rearrangement because the longitudinal
differentiation (banding) of the specific chromosome is
lost and probes to paint specific portions of chromosomes
are not available by this method. Therefore, the
approach is not effective in identifying either break
10   point sites in rearrangements or deletions (or other
events in which only a single chromosome is affected).

Another way to isolate human chromosome-specific DNA
is amplification (by polymerase chain reaction -- PCR)
15   from interspecific hybrid cells containing only the human
chromosome or chromosomal region of interest. Human-
rodent hybrid cells monochromosomal for virtually every
human chromosome and for portions of a human chromosome
are now available. Since it is known (15) that the human
20   genome has hundreds of thousands species-specific repeat
sequences scattered throughout, several groups (16-19)
have prepared consensus primers to bind to these
sequences. By using these primers for PCR amplification,
some success in pulling out human chromosome specific DNA
25   sequences from hybrid cells and various types of
recombinant DNA libraries have been achieved. However, a
recently reported attempt to make painting probe from
such material (20) resulted in speckled chromosomes with
high background with no possibility of observing any
30   longitudinal differentiation of the painted genetic
element. Although selective in amplifying human DNA from
hybrid cells, sensitivity in chromosome painting was low.
Thus, the reported methods are impractical for developing
probe useful for the identification of deletions or
35   translocation breakp ints in abnormal cells.

An attempt to paint specific human chromosomes with
the total DNA from a hybrid cell containing the human
chromosome of interest has met with partial success (21).
Unfortunately, for most of the chromosomes attempted, no
5   clear specific painting was obtained. In the few cases
where success in painting a specific chromosome was
achieved, the entire chromosome was painted and there was
no possibility of observing longitudinal differentiation.

10   Identification of cells having chromosomal
rearrangements at known breakpoints on the affected
chromosomes has been accomplished by using, as probe,
sets of cosmids that flank the breakpoint. Use of such
cosmid probes flanking the breakpoint region of the p-arm
15   of chromosome 16 has enabled visualization of the
inversion associated with acute nonlymphocytic leukemia
(22). However, the intensity of the signal was
relatively weak. One would expect a much stronger signal
if the entire arm of the chromosome were painted. Using
20   a similar technique, the CML Ph chromosome has been
identified using a pair of cosmids, one from the brc gene
proximal to the breakpoint on chromosome 22 and the other
from the abl gene distal to the breakpoint on chromosome
9 (23). The intensity of the signal appeared weak,
25   raising doubt that it could be reliably used to identify
the breakpoint in the majority of cells without computer
enhancement. These latter two approaches, while somewhat
effective for chromosomal alterations where genomic
regions flanking the breakpoints have been cloned, are
30   useful only where such sites have been precisely
identified or isolated. In the vast majority of cases,
the sites are unknown and there is no effective method of
identification.

35   Finally, DNA probes have been developed that
specifically detect the centromeres (the dot-like

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structure at the junction of the two chr mosome arms) for
particular chromosomes (24). These probes are supposed to
be effective in determining the number of chromosomes
(for chromosomes from which such probes have been
5   developed) in cells (24). However they not only provide
no information on rearrangements involving those
chromosome but often lack specificity in that probe
developed for the identification of one centromere will
often cross-hybridize to centromeres of other
10   chromosomes.

    Therefore, progress has been made in developing
techniques to selectively identify individual human
chromosomes. However, current methods have several
15   shortcomings, including: (1) lack of detection of
abnormal chromosomes in less than ideal metaphase
spreads; (2) impracticality in determining the frequency
of abnormal cells in a complex tissue; (3) inefficient
detection of identify of marker chromosomes; (4) lack of
20   specificity in identifying sub-chromosomal regions; (5)
great time and expense involved in either karyotyping or
preparing probe; (6) lack of flexibility; and (7) failure
to paint chromosomes adequately while still observing
landmarks for longitudinal differentiation.
25
    The present invention is designed to address these
problems. It provides a highly efficient and selective
means for identifying individual chromosome
abnormalities, as well as marker chromosomes in
30   interphase as well as metaphase nuclei; has the
flexibility to develop probe for specific chromosomal
regions; and permits the efficient manufacture of probes
that will not only brightly paint a human chromosome of
interest, but will also paint on that chromosome a
35   banding pattern that will enable the identification of
th sites of chromosomal aberrations.

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The invention is a method to prepare DNA probes that are specific for virtually any desired chromosome or part of a chromosome. A novel aspect of the probes is that they are relatively free of repeat sequences so that a
5   probe directed to a particular chromosome or part of a chromosome will hybridize only with that chromosome or part of a chromosome. The probe will not hybridize to other chromosomes of the same species because the probe will have esssentially only unique DNA sequences of the
10   desired chromosome.

In general, chromosome specific DNA probe may be prepared by first isolating the desired DNA and then amplifying only regions of chromosome specific DNA. This
15   is achieved by hybridizing primers to certain regions of a species-specific repeat sequence, then amplifying. The DNA between the repeat segments is amplified, but little if any of the repeat segment is synthesized. The resulting DNA probe is essentially free of species
20   specific repeat DNA sequences to which the primer was hybridized.

In the practice of the invention, one should determine or select the particular a chromosome or part
25   to which probes are desired. The chromosome may be selected from any number of sources, including insects, birds, fish, humans, animals or any living thing that has a chromosome with chromosome-specific DNA sequences and a sufficient number of interspersed segments with species
30   specific repeat DNA sequences. Mammals, including humans, have such interspersed segments, for example, LINES or SINES. Clustered repeat sequences are also found in some species, for example the alphoids found in humans. One type of SINE found in mammalian species is
35   the Alu repeat sequence. The chr m some, r portion thereof, need not be free of chr mosomes from other

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species as long as its species specific segments are
different enough in sequence from the repeat segments of
the other species so that primers for polymerase chain
reaction (PCR) may be prepared which will selectively
5   bind to the sequences of the repeat segments associated
with desired species.

DNA for use in the preparation of probes may be
obtained from the any source in which the chromosome, or
10   portion thereof, has been isolated from the remainder of
that species other chromosomal material. These sources
include interspecific somatic cell hybrids, flow-sorted
preparations, microbial vector clones, or microdissected
DNA. In a most preferred embodiment the chromosome or
15   portion thereof to which probe is being prepared is
isolated in interspecific somatic cell hybrids, such as
hamster.

In the practice of the invention, it is typical to
20   prepare two primer sets for use in subsequent PCR
amplification, each set having at least one primer which
is capable of hybridizing to a region within a repeat
segment. One or more primers from the first primer set
will preferably bind at or near the 5' terminus of such
25   repeat segments within the chromosome or chromosome
fragment. The primer or primers will facilitate DNA
replication in the direction of the repeat DNA segment 5'
terminus. Similarly, one or more primers from the second
set of primers will preferably bind at or near the 3'
30   terminus of a repeat segment and will facilitate DNA
replication in the direction of the 3' terminus.

After the primers are prepared, an annealing step is
performed by hybridizing with DNA obtained from the
35   material c ntaining th chrom s mal region f interest.
Annealing by hybridization techniques is well known to

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those skilled in the art and generally is conducted at a
temperature between about 50°C and 60°C. In an important
aspect of the present invention, when interspecific
somatic cell hybrids are the source of the chromosomal

5   material for which probe is being made, hybridization is
performed at a higher temperature, in the range of 60°-
70°C, most preferably at 65°C. This prevents annealing
of the primers to repeat segments in chromosomes of the
other species.

10

A final step in preparing chromosome specific DNA
probes is amplification of the inter-repeat regions of
the selected chromosome or chromosome part. For human
chromosomes, the DNA amplified will be specific for that

15   particular chromosome or part. Since DNA synthesis is
directed away from the middle and off the termini of
human Alu repeat segments, the DNA probe so produced will
contain a minimum amount of Alu repeat sequences. A
small amount of Alu repeat sequence amplified may arise

20   because the primer sets do not bind to the very ends of
the Alu repeat segments.

In humans as well as other mammals, species specific
repeat sequences occur frequently. Segments of Alu

25   repeat sequences are the most prevalent middle repeat
elements in human chromosomes. When the method is used
to prepare human chromosome or human chromosome portion-
specific DNA probes, the first primer set (Alu-1) is
designed to bind to base pairs 13-31 from the 5' end of

30   300 bp Alu segment. In a preferred embodiment, primers
in this first primer set have a sequence which is reverse
complementary to the Alu consensus sequence located from
base pair 13-31. This means that priming with Alu-1 will
direct DNA synthesis toward the 5' terminus, and away

35   from the middle, of the Alu segment in which the primers
bind. In a m st preferred embodiment, complementarity to

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the consensus sequence is sufficiently degenerate so that
binding to a large number of human Alu segments occurs.
This is accomplished using a set of primers synthesized
so that, in combination, the primers will bind to
5   virtually all Alu segments. In this embodiment, the
primers do not bind with Alu segments of nonhuman
species.

     In a most preferred embodiment, members of the Alu-1
10   set have the base sequence GGATTACAGGYRTGAGCCA. Y is
either pyrimidine T or G and R is either purine A or G.
The Alu-1 primer set most preferably comprises four
primers with either thymidine or cytosine at position 11
and adenine or guanidine at position 12. In a most
15   preferred embodiment the primers are used in
approximately equal amounts. These primers will bind to a
large number of human Alu consensus segments but not to
nonhuman Alu segments. It will be appreciated by those
of skill in the art that other primers may be effective
20   in the practice of the invention, such as somewhat
shorter or longer primers. Also, fewer than four primers
may be used. The closer the primer binds to the 5' end
of an Alu 300 bp repeat segment, the less Alu repeat
sequence DNA will be obtained on subsequent amplification
25   of chromosome specific DNA. Although binding at the very
end of human Alu sequences is preferred, Alu sequences
are less conserved at that position and a sufficiently
consensus sequence is not identified. It is preferred
that the primer bind to as many Alu regions as possible
30   in order to promote amplification of as much chromosome
specific DNA as possible. It should also be appreciated
that repeat segments other than Alus occur in human
chromosomes. Analogously designed primer sets could be
used with the Alu primers to eliminate essentially all
35   repeat segments.

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A second primer set (Alu-2) has hat least one primer
and is also designed to bind with a human Alu consensus
sequence, but these primers will preferably bind at or
near a 3' terminus of a 300 bp Alu segment, not at the 5'
5   end. The primers in Alu-2 have a base sequence identical
to a base sequence within an Alu repeat segment and
therefore prime to direct DNA synthesis in a 3'
direction, that is, away from the center of the 300 bp
Alu segment to which they are annealed. A preferred
10   consensus sequence is located within 100 base pairs of
the 3' terminus, most preferably at base pair position
240-258 of 300 bp Alu repeat segment. The primers in
Alu-2 most preferably have base pair sequences that bind
with the majority of human Alu repeat segments. This is
15   accomplished by taking into account the degeneracy of
consensus sequences within Alu segments and preparing a
mixture of primers with base sequences which bind with
nearly all the consensus sequences. The primers in the
Alu-2 set comprise primers having the base sequence
20   RCCAYTGCACTCCAGCCTC where R is A or G and Y is T or C. A
most preferred number of primers in the second primer set
is four and they are most preferably used in
approximately equal amounts.

25   In a particular aspect of the invention, human
chromosomes or parts are used to prepare specific probe.
For example, any of the 24 different human chromosomes
(number 1-22, the X and the Y) may be isolated in various
hybrid cells and used to prepare probe specific to that
30   chromosome by the described method. Additionally, a
portion of a chromosome may be used, for example, the p-
arm of chromosome 16, band q13.3 of chromosome 19, 1q,
3q, 5q, 7q, 9q or 22q. The method has been demonstrated
with portions of a chromosome as small as 1-2 Mb of human
35   DNA from band q13.3 of human chromosom 19. The lower
limit of detection may be as low as 500 base pairs in

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situations where specific DNA bracketed by repeat
sequence is obtained and amplified as probe by the method
described.

5   DNA of the desired chromosome may be isolated from
species total chromosomal material by flow sorting or,
preferably when chromosome parts are desired, from
interspecific somatic cell hybrids or artificial yeast
chromosomes. Isolation may also be by microdissection of
10   the desired DNA.

The invention also relates to a method for painting
a human chromosome or portion of a human chromosome in
metaphase spreads or interphase nuclei. The DNA probes
15   prepared are specific for the chromosome or part
selected. The probes are then labeled with a substance
that can be later detected, for example, biotin,
digoxigenin, ^{32}P, dinitrophenol, aminoacetylfluorene or
conjugated mercury, and incubated with a sample in which
20   the human chromosome or portion is to be identified by
in-situ hybridization, conjugated, for example, with
avidin-fluorescene, Texas red, fluorescein isothiocyanate
or acid phosphatase and detected by one of a series of
methods such as fluorescence, autoradiography or
25   chemoluminescence. These are procedures well known to
those skilled in the art. Biotin is the preferred label,
avidin-fluorescene the preferred conjugant, and
fluorescence the preferred detection method.

30   Small amounts of amplified repeat sequences
amplified from the human chromosome of interest and which
are not chromosome specific may be prevented from
participating in the *in-situ* hybridization reaction. In
another innovative aspect of the invention, this is
35   accomplished by blocking those non-chromosome-specific
regi ns with middle- and highly- repetitive DNA isolated

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from the total DNA of the species, most preferably Cot 10
DNA.

The invention also includes a method of removing
5   small amounts of amplified repeat sequence DNA from the
in-situ hybridization probe using PCR amplified Alu
repeat sequence terminal regions. Plasmid comprising a
complete 300 bp species specific repeat Alu sequence is
used, preferably Blur2. Two primers are then prepared
10   that will anneal each of the two plasmid arms. These
primers, and Alu-1 or Alu-2 primers are annealed to DNA
from the plasmid. PCR is conducted in order to amplify
the repeat sequences from the two ends of the Alu segment
(bp 1 - bp 31 and bp 240 - bp 300). The amplified Alu
15   terminal regions are linked to a carrier, then the
labeled Alu sequences from the probe DNA are hybridized
to them. This removes the terminal Alu repeat sequences
from the probe to be in-situ hybridized. A preferred
carrier useful for linking with amplified Alu terminal
20   regions is diazobenzoxymethyl cellulose although other
carriers such as diazotized plastic beads may also be
used. Where repeat sequences are prevented from
participating in the in-situ hybridization reaction by
blocking, the efficiency of that blocking reaction can be
25   increased by the addition of the amplified Alu terminal
regions to the middle and highly repetitive DNA used for
such purpose.

The chromosome painting method may also be applied
30   to painting of R-banding patterns on specific human
chromosomes in metaphase spreads. Good metaphase spreads
are not critical.

The present invention also contemplates the use of
35   human Alu primer s ts and sp cific chromosome pr bes in
kit form. Thus in a preferred mode of use, lyophilized

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human specific inter-Alu-1 and inter-Alu-2 primers could
be used to conveniently amplify a selected human
chromosome or portion of human chromosomes to produce DNA
probe directed to that particular DNA. In yet another
5   preferred mode of use, labeled DNA probe specific for
selected human chromosomes would be provided to use as
probe. This could include DNA probes for human
chromosomes 1-22 and the x and y chromosome and also
chromosome parts, such as 16p, 19 q13.3, 9q, 5q, 7q, 3q,
10   1q and the like.

The invention also includes two unique primer sets
each comprising four primers especially designed for use
in the production of human specific probe for any desired
15   chromosome or part thereof. Each set may comprise one or
more primers but is most preferably four primers. The
base sequence of one primer set is GGATTACAGGYRTGAGCCA
and the base sequence of the second primer set is
RCCAYTGCACTCCAGCCTG where Y represents T or C and R is A
20   or G. There are four primers in each set and in a most
preferred mode of use are used as mixtures of
approximately equal amounts.

Figure 1 shows the agarose gel separation of inter-
25   Alu-PCR products using both Alu-1 and Alu-2 primers
(1/2), Alu-1 primer only (1), or Alu-2 primer only (2) on
HeLa DNA and CHO DNA. Unmarked channels are size
markers.

30   Panel A. (Left). PCR conducted under standard
conditions, annealing temperature 55°C.

Panel B. (Right). PCR annealing temperature at
65°C.
35

Figure 2 shows an agarose gel separation f inter-

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Alu-PCR products after using the Alu-1/Alu-2 primer sets
(O) or a published Alu PCR primer (N) on human genomic
DNAs from a series of cosmids (numbered 1-5).

5   Figure 3 is a photomicrograph of a metaphase from
hybrid 5HL9-4, which contains human chromosome 19 as the
only human genetic element, in-situ hybridized using
total human DNA as probe. The arrow indicates the only
fluorescing chromosome.

10

        Figure 4 is a photomicrograph of a human metaphase
spread after in-situ hybridization with inter-Alu-PCR
probe obtained from the hybrid containing human
chromosome 19 as the only human genetic element. Arrows

15   indicate two fluorescing chromosomes, identified as the
human 19 chromosomes. Note the absence of fluorescence
at the centromeres (tips of the arrows).

        Figure 5 is a photomicrograph of a metaphase of a

20   hybrid cell (41XP91-3) containing the p-arm or human
chromosome 16 as the only human genetic element following
in-situ hybridization with total human DNA. The arrow
indicates one small fluorescing fragment containing human
DNA.

25

        Figure 6 is a photomicrograph of a human metaphase
spread following in-situ hybridization with inter-Alu-PCR
probe made from the hybrid (41XP91-3) containing the p-
arm of human chromosome 16 as the only human genetic

30   element. Arrows indicate both p-arms of human chromosome
16 as the only fluorescing elements.

        Figure 7 is a photomicrograph of metaphase following
in-situ hybridizati n with total human DNA on a hybrid

35   cell line (2F5) containing from 1 - 2 Mb of human DNA
from the q-arm of human chrom som 19 as the only human

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genetic element. That element is translocated onto a CHO
chromosome. A small region of fluorescence on an
otherwise non-fluorescent chromosome is indicated by the
arrow.

5

   Figure 8 is a photomicrograph of two human metaphase
cells following *in-situ* hybridization with inter-Alu-PCR
probe made from hybrid 2F5. Arrows indicate the location
of narrow bands of fluorescence appearing only on both

10   chromosome 19 q-arms.

   Figures 9 and 10 are photomicrographs of two human
metaphases following *in-situ* hybridization with inter-
Alu-PCR probe made from the hybrid containing only human

15   chromosome 7. The arrow indicates the location of two
chromosomes with fluorescence in each cell. The
fluorescence produces an R-banding on the chromosomes.

   Figure 11 is a photomicrograph of a human metaphase
20   following *in-situ* hybridization with inter-Alu-PCR probe
made from DNA of a hybrid containing chromosome 5 as its
only human genetic element. Note the presence of only
two fluorescing chromosomes in the cell with the broad R-
band pattern distinctive for human chromosome 5.

25

   Figures 12 and 13 are photomicrographs of human
metaphases following *in-situ* hybridization with inter-
Alu-PCR probe made from a hybrid containing chromosome 17
as its only human genetic element.   Both chromosome 17s

30   are lit up in both figures. Where the chromosome if more
condensed (figure 12) the R-bands are closer together and
the R-banding pattern is not obvious. The chromosomes
are identified in poorly spread metaphases as shown is
Figure 13.

35

   Figure 14 is a photomicrograph of a human metaphase

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following *in-situ* hybridization with inter-Alu-PCR probe
made from a hybrid containing chromosome 3 as its only
human genetic element. Both chromosome 3s are the only
fluorescing elements in the broad R-band pattern, with a
5   dark centromeric region characteristic of human
chromosome 3.

Figure 15 is a photomicrograph of a human leukemic
cell with a known chromosome 16 inversion after *in-situ*
10 hybridization with inter-Alu-PCR probe made from a hybrid
containing only the p-arm of chromosome 16. At the
bottom of the field is the unaffected chromosome 16 with
only the p-arm fluorescent. Note the inversion
chromosome with fluorescence not only the end of the p-
15 arm but also in the middle of the q-arm (arrow).

Figure 16 is a photomicrograph of a metaphase from a
leukemic cell line with a known chromosome 7 deletion
following *in-situ* hybridization with the inter-Alu-PCR
20 probe made from a hybrid cell containing human chromosome
7 as its only human DNA content. In addition to a banded
fluorescing intact chromosome 7, a second banded
fluorescing chromosome 7 deleted distal to the bright
band on the proximal region of the long arm is shwon
25 indicated by the arrow.

Figures 17 and 18 are photomicrographs of normal
human interphase nuclei following *in-situ* hybridization
with the inter-Alu-PCR probe made from hybrid 2F5 which
30 contained only 1 - 2 Mb of human DNA from band q13.3 of
human chromosome 19. Notice the bright, punctate
fluorescence resolving each of the domains occupire by
these regions of chromosome 19 in the interphase cells.

35 Figure 19 is a photomicrograph of an interphase
nucleus of a human leukemic cell with a known chromosome

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16 inversion after *in-situ* hybridization with inter-Alu-
PCR probe made from a hybrid containing only the p-arm of
chromosome 16. Two bright domains of hybridization are
interrupted by non-fluorescence as indicated at the
5   arrow.

       The invention relates generally to the preparation
of human chromosome specific probes that are essentially
either blocked for or free of non-chromosome specific
10   repeat sequences. These probes when applied to human
cells via the process of *in-situ* hybridization, will
identify the human chromosomal region of interest. The
procedure will allow the rapid identification of
chromosomal abnormalities associated with various forms
15   of cancer and birth defects.

       A novel aspect of the invention is the design,
preparation and conditions for use of two sets of
consensus Alu-PCR primers for manufacture of the
20   chromosome specific probes by inter-Alu-PCR. The scheme
takes advantage of the fact that Alu segments are
distributed throughout the human genome and that by using
them to instigate PCR reactions, unique, chromosome-
specific sequences located between such segments may be
25   amplified for use as probe for chromosome identification.
Alu sequences are of the most abundant family of middle
repetitive DNA sequences in the human genome. About
500,000 Alu family members are present in the haploid
human genome, amounting to about 6% of the total mass of
30   the DNA (25). These Alu sequences are not precise copies
of a single sequence but instead are related copies of a
consensus sequence which is approximately 300 base pairs
(bps) long and consists of two monomer units.

35   Alu primers were designed based n several
considerations. First, it was necessary to insure that a

-20-

maximum number of Alu sequences were annealed by the
primers. It is estimated that the average divergency of
Alu sequences from the consensus is 12% (26). This
divergency (or degeneracy) needs to be considered in the
5   design of the primers in order for them to anneal with as
many Alu sequences as possible. Second, since the Alu
sequences themselves are not chromosome specific, it was
necessary to insure minimum representation of the Alu
sequence themselves in the product of the inter-Alu-PCR
10   without impairing the first consideration. Therefore,
the primers had to be designed to recognize the ends of
Alu segments and direct priming away from the middle of
such segments. Third, the primers needed to be designed
so that inter-Alu-PCR would occur independent of the
15   orientation of Alu segments in the human DNA to maximize
the amount of unique sequence amplification. Finally,
the primers needed to be human specific in order to
amplify selectively human DNA in the presence of excess
DNA from other species.
20

     The Alu primers were designed based on a current
revision of consensus sequence of human Alu repeats (25).
The revision was based on nucleotide sequences of 50
different, cloned and sequenced human Alu segments. Two
25   regions on the sequence showing high degree of
conservation among Alu family members were identified.
One is at bp 10-50 and the other at bp 240-260. These
were the candidate regions for the primer locations. One
provided the basis for design of the first primer (Alu-1)
30   that bound near the 5' end of a 300 base pair (bp) Alu
segment, and a second stretch was used to design the
second primer (Alu-2) which bound near the 3' end.

     In considering the first or 5' region, it was
35   n ticed that there was a lack f sequence conservation
around p sition 20. At p sition 20, 31% f the segments

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had a T rather than C; and at position 21, 42.5% of the
Alu sequences had an A instead of G. In order to
maximize binding of primers at this region, degeneracy at
these two positions was taken into consideration in
5   primer design. Bp 10-12 could not be used because there
were substitutions of more than one nucleotide at those
positions. Therefore, the Alu sequence selected for the
design of the Alu-1 primer went from bp 13 to bp 31 and,
taking degeneracy into consideration, had the sequence
10   TGGCTCA(C/T)(G/A)CCTGTAATCC. In order to minimize the
incorporation of Alu sequence itself in the product of
the inter-Alu-PCR, the primer was designed to recognize
that region and to direct DNA synthesis off the 5' end
and away from the middle of the Alu segments to which it
15   bound. Therefore, the actual Alu-1 primer is the reverse
complement of that sequence or GGATTACAGGYRTGAGCCA (where
Y is either pyrimidine, C or T; and R is either purine, A
or G).

20   For the second or 3' region there were regions of
degeneracy at bp positions 240 and 244. At bp 240, 37.5%
of the Alu members had an A instead of G. At position
244, 32.7% had a T instead of a C. For the same reason
as above, degeneracy was incorporated into the primer
25   design and the sequence of primer Alu-2 is
RCCAYTGCACTCCAGCCTG. This is a direct and not an inverse
complementary sequence directing priming off the 3' end
of an Alu segment.

30   Since each primer reads away from the middle of the
Alu segment, the resultant inter-Alu-PCR will not only be
initiated from a maximum number of Alu segments but also
incorporate a minimum amount of Alu sequence.
Furthermore, the design takes into consideration the fact
35   that tw adjac nt Alu s quences could be arranged head to
head, tail to tail r in tandem. Having one primer at

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each end with the direction of DNA synthesis away from
the Alu segment enables amplification between any two Alu
sequences independent of their orientation when the two
Alus are within appropriate distance for PCR. This
5   yields product ranging from a few hundred to several
thousand base pairs. Therefore, the primer design
maximizes both the number of Alu segments recruited and
the number of inter-Alu unique sequences amplified while
minimizing the amount of Alu sequence incorporated into
10   the reaction products and defines the DNA probes as
practiced by this invention.

Comparing Alu-1 and Alu-2 with rodent Alu-like
consensus sequences (27) indicated that 11 nucleotides at
15   the 5' end of Alu-2 are not present in either Chinese
hamster or mouse. For Alu-1, there are 7 mismatches in
Chinese hamster sequence and there is no corresponding
sequence in mouse. Therefore, it was possible to
establish conditions for the specific amplification of
20   human DNA using these two primers in the presence of
rodent DNA. Thus, when there is only one, or part of
only one, human chromosome in the DNA from a human x
rodent cell line subjected to the PCR, the amplified DNA
will be specifically from that human chromosomal region.
25   This material can then be used for in-situ hybridization
to identify the location of those unique sequences in the
human genome.

As a second innovative feature, this method is
30   designed to be used on DNA from interspecific somatic
cell hybrids that retain only a single, or part of a
single, human chromosomal element isolated against a
rodent chromosomal background. By conducting the PCR
reaction, under conditions described below, on the DNA
35   from such cells using the primers designed above, only
the human DNA (repres nting the human chromosomal

material on interest in the hybrid cell) will be
manufactured in large quantity by PCR amplification.

    In this procedure, the temperature conditions for
5   the crucial annealing step of PCR are elevated above the
    standard temperature used for the reaction because
    variations of the consensus sequences recognized by the
    primers (Alu-1 and Alu-2) also exist (as indicated above
    -- 27) in the rodent versions of Alu segments (Alu
10 sequences have some level of evolutionary conservation in
    mammalian species). At standard temperatures of
    annealing, the primers may bind to rodent Alu sequences
    of shared homology, thus decreasing specificity toward
    human chromosomal material present in the hybrid cell.
15 However, at higher temperatures, the sequences of the
    primers and the human Alu sequences to which they are
    designed to bind, must be more precisely matched allowing
    the Alu primers designed to recognize consensus human Alu
    sequences to amplify only the human chromosomal material
20 present in the DNA.

    For this amplified DNA from a specific chromosomal
    region to function effectively as probe to identify that
    specific chromosomal region in a human cell, repeat
25 sequences present in the DNA must be prevented from
    taking part in the *in-situ* hybridization reaction for
    which the probe material will be used (8,9). This is
    necessary since, in addition to the chromosomal-specific
    unique sequences, the inter-Alu-PCR reaction will amplify
30 Alu repeat sequences located at the ends of the Alu
    segment sites of priming as well as other types of non-
    chromosomal-specific repeat sequences which might be
    located between Alu segments participating in the
    reaction. Since such repeat sequences are not specific
35 for th chromosomal regi n of interest, in the *in-situ*
    hybridization r action, they will hybridize to

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chromosomal regions on other human chromosomes. These
repeat regions were effectively prevented from
participating in the reaction by designing a blocking
agent made from total human DNA. This was accomplished
5   with high efficiency isolation of a repeat sequence-rich
fraction of human DNA (called low-Cot DNA) and
prehybridizing to probe DNA. This effectively blocked
the participation of the repeat sequence regions in the
in-situ hybridization reaction and gave the probe its
10   specificity to the chromosome region of interest.

Another important unique feature of this approach is
that preparing probe in this manner (inter-Alu-PCR)
results in probe that not only binds specifically to the
15   human chromosome of interest but will also impart to that
human chromosome a banding pattern. This makes it
possible to identify regions where the normal chromosome
structure has been modified (by deletion, translocation
or inversion). The observed banding pattern is resolved
20   because Alu sequences are not homogeneously distributed
throughout the human genome but rather are in higher
concentration in specific regions (R-bands) along the
length of the chromosomes (28). Consequently, probe made
by inter-Alu-PCR from a human chromosome DNA will bind
25   preferentially, not only to the human chromosome from
which it was derived, but also to the longitudinally-
specific regions of that chromosome that are rich in
Alus. Therefore, the procedure will not merely paint the
human chromosome of interest, but will paint on the
30   chromosome the specific banding pattern unique to it.

Since the centromeric regions of chromosomes are
rich in the highly repetitive alphoid repeat sequences
(which are a different class f repeat sequence than Alu
35   ---24), they are not amplified by the inter-Alu-PCR.
Consequently the centromeric r gions of the chr mosomes

are not painted by probe made by this method (e.g.,
Figure 4). Painting probes which include centromeric
regions are often not chromosome specific because those
non-chromosomal specific regions are not readily blocked
5 (due to the *highly* repetitive clustered nature of such
sequences, as opposed to the *middle* repetitive status of
the interspersed Alu repeats) or eliminated from the *in-
situ* hybridization reaction (12,13). Eliminating such
regions in the design of our probes enhances their
10 chromosomal specificity.

One of the special advantages of making *in-situ*
hybridization probe for specific chromosome regions by
inter-Alu-PCR from hybrid cells containing only those
15 regions of the human genome is the flexibility provided
by being able to prepare probe that identifies only
chromosomal arms, regions or sub-regions. Such probe can
readily identify chromosomal abnormalities involving only
a single chromosome (inversions or deletions) as
20 indicated in Example 8.

In addition chromosome region-specific probes make
it possible to identify and resolve those regions in an
interphase nucleus (the compartment of the cell cycle in
25 which upwards of 90% of the cells are located). In
interphase, the chromosomes are not visible because they
are not condensed. It is a well recognized phenomenon
that *in-situ* hybridization probe for a specific
chromosome will identify the domain that chromosome
30 occupies in an interphase nucleus (8,9). Since
chromosomes are "unwound" in that phase, entire
chromosomes present very large, unresolved domains of
hybridization. By being able to make probe from only a
small fraction of a chromosome, the domain visualized
35 becomes smaller and better resolved. The id ntification
of chromosomes and chr mosomal abn rmalities in such

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nuclei then becomes possible. The demonstration of such
resolution using such probes and indication of their
usefulness in observing intra-chromosomal abnormalities
in interphase nuclei is cited in Example 9.

5

However, the identification of _translocations_ in
interphase nuclei by using combinations of two such
probes tagged so that they will fluoresce in two
different colors and which are derived from small regions
10   of the affected chromosomes flanking the translocation
breakpoints will be the major application of inter-Alu-
PCR probes to interphase nuclei. The translocation will
be observable as a combination of the two colors in those
nuclei. Since these chromosome regions, from which
15   probes are made by the invented method, are measured in
millions of base pairs in length the signal they give
will be quite outstanding and observable in every cell
nucleus without the need for computer enhancement (as in
Example 9). This distinguishes them from present
20   methodology in which human cosmid clones flanking the
translocation breakpoint sites are labeled with different
color probes and used to observe the translocations in
interphase nuclei (23). Since the cosmid clones are only
tens of thousands of base pairs in length, the signal is
25   weak and not reliably visualized in every interphase.

This ability to reliably determine if the
translocation chromosome is present in interphase nuclei
will be of major importance in the management of minimal
30   residual disease during cancer chemotherapy. In such
cases one wishes to determine the low frequency of cells
in the bone marrow of a leukemia patient that had, or is
undergoing, treatment. Since that frequency is low
(perhaps 1/1000 or 1/10,000) it is impractical to make
35   the determinati n from only the few cells in metaphase
that one recovers from such pr parations. It is also

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likely that the cells in metaphase might be a
disproportionate representation of a single cell type and
not representative of the population of cells as a whole.
The ability to see the translocation in the interphase
5   cell will not only be an excellent monitor for the
effectiveness of treatment but also be an extremely
useful research tool in determining the etiology of the
disease.

10   While the procedure was designed for preparing *in-
situ* hybridization probe by amplifying human chromosomal
regions isolated in hybrid cells, the inter-Alu-PCR
method will also be effective in providing probe from
chromosome regions isolated by other methods -- flow-
15   sorted human chromosomes (8,9); chromosome regions cloned
into large insert microbial vectors such as yeast
artificial chromosomes (YACs -- 29); or chromosome
regions scraped by microdissection from a microscope
slide (30). In these cases it will not be necessary to
20   increase the annealing temperature for the PCR reaction
since there is no danger of amplifying contaminating
(non-human) DNA.

         While the innovative procedure for blocking repeat
25   sequence DNA from participating in the *in-situ*
hybridization reaction is effective, the present
invention also includes a method to completely remove
repeat sequences. Therefore, the hybridization reaction
may be conducted with pure unique sequence specific to
30   the chromosomal region of interest. As indicated above,
there are two sources of repeat sequence in the inter-
Alu-PCR probes that require elimination. The first
source is present because of the fact that primers Alu-1
and Alu-2 are not located at the very ends of Alu
35   segments (the v ry ends, although still Alu, are not
sufficiently conserved to be able t identify a useful

consensus sequence for use as primer). Therefore all
amplification products will have at least 62 to 120 bp of
terminal Alu sequences in them (depending upon whether a
segment was the amplification product of priming from
5   Alu-1 to Alu-1, Alu-1 to Alu-2, or Alu-2 to Alu-2). The
second species of repeat sequences present in probe are
those repeat sequences which happen to be located between
Alu sequences which, along with the desired unique
sequence, get amplified as a result of the inter-Alu-PCR.
10 In order to more effectively block the Alu ends, Alu-1
and Alu-2 primers are to be used in PCR reactions with
primers that recognize the arms of a plasmid containing
the complete Alu sequence (BLUR2 -- 31). This will
amplify those same terminal Alu sequences that were
15 amplified by the inter-Alu PCR. Those amplified terminal
Alu sequences, along with low-Cot DNA (described above
and which represents additional Alu segments and other
species of repeat sequences present in the inter-Alu-PCR
product), may be covalently linked to
20 diazobenzyloxymethyl cellulose (32,33) and hybridized
with the labeled probe (34,35) following nick-translation
and biotinylization. Biotinylated fragments containing
the repeat sequences will hybridize to those sequences
bound to the cellulose and may be centrifuged out.
25 Remaining biotinylated probe (containing only chromosome-
specific unique sequences) will then be directly
hybridized to metaphase spreads to detect the specific
human chromosome regions of interest. An economical
feature associated with the use of the diazonium
30 cellulose with the chemically bonded repeat sequences is
that the biotinylated fragments, attached to them after a
reaction cleaning up the inter-Alu PCR reaction, can be
removed by simply reheating the complex. This
regenerates the diazonium cellulose with its bound repeat
35 sequences for reuse in cleaning up more inter-Alu-PCR
product.

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While this invention has been described in terms of
providing probe for specific regions of the human genome
so that those areas can be visualized by *in-situ*
hybridization in order to detect any possible alteration
5   of those regions in cancer cells or cells of a developing
embryo, such probe may also be used to isolate specific
human genes located in those specific chromosomal
regions. This is done by using probe made by any of the
options above to screen tissue specific cDNA libraries.
10   cDNA libraries are recombinant DNA molecules packaged
usually into phage hosts in which the human DNA
represents the human genes transcribed in those
particular tissues. This technique will be extremely
useful when genetic studies reveal, for instance, that
15   the gene for Alzheimer's disease is located on a specific
region of human chromosome 19. Then by making inter-Alu
PCR probe for that specific region of human chromosome
19, brain cDNA libraries could be screened to isolate the
human genes from that region for identification of
20   candidate genes for the disease.

<u>Materials and Methods</u>

<u>Preparation of DNA template from hybrid cells for inter-</u>
25   <u>Alu PCR</u>

DNA template from hybrid cells may be prepared by
several methods, two of which are described. The first
method is most preferred in the practice of the
30   invention.

1.   <u>DNA in Solution</u>. DNA was isolated in traditional
fashion by methods well known to those skilled in the art
and are as described (36).
35

2.   <u>DNA in Agarose Plugs</u>. Preparation of genomic DNA in

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agarose plugs was performed according to van Ommen and
Verkerk (37). For PCR, the plugs were washed in 10 mM
Tris-HCl pH7.5 for four changes at room temperature, 15
minutes each, then melted at 65°C for 5 minutes. 10 mM
5   Tris-HCl pH7.5 was added to a final volume of 500 μl.
The size of the DNA was reduced by vortexing for 30 sec.
before storage at 4°C. 10 μl was used for each PCR.
This method also gave satisfactory results but was less
preferred to method one above.
10

PCR protocol

The consensus inter-Alu-PCR primers designed were:

15   Alu-1:   GGATTACAGGYRTGAGCCA, and

        Alu-2:   RCCAYTGCACTCCAGCCTG

where Y is either pyrimidine (T or C) and R is either
20 purine (A or G). Therefore the Alu-1 primer is actually
a cocktail composed of the 4 possible Alu-1 molecules (T
at bp 11 and A at bp 12; T at bp 11 and G at bp 12; C at
bp 11 and A at bp 12; and C at bp 11 and G at bp 12).
Similarly Alu-2 is a cocktail of the 4 possible Alu-2
25 molecules with all possible combinations of alternative
purines and pyrimidines at positions 1 and 5
respectively.

In a final volume of 50 μl, was added 100 ng genomic
30 DNA in solution or 10 μl of melted agarose plug, 5 μl of
10 x PCR buffer (100 mM Tris-HCL, pH 8.3, 500 mM KCL, 15
mM $MgCl_2$ and 0.01% gelatin), 0.2 mM dNTP, 50 pmoles of
each of the two primers, and 1.25 units (0.25 μl of 5
unit/μl) Taq polymerase from Perkin-Elmer Cetus. The PCR
35 reaction was conducted using a thermal cycler from
Precision Scientific, Model GTC-1. After an initial 5

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min incubation at 94°C, samples were subjected to 25
cycles as follows: 94°C 1 min, 65°C 1 min (a higher was
used instead of the standard annealing temperature of
55°C in order to amplify only human sequences in the
5   presence of other mammalian Alu sequences, such as in a
hybrid cell -- for PCR from YACs, phage or any source
where human Alus are the only ones present the standard
annealing temperature would be preferable), and 72°C 3
min. Finally an elongation of 10 min at 72°C was
10 conducted. One-tenth aliquots of each sample were run on
gel. Primers and free nucleotides were removed with
Centricon-100 (Amicon).

Evaluation of PCR product on agarose gels

15
        500 ng of PCR product was loaded on a 0.8% agarose
gel (7 cm x 7 cm) and run at 80 - 100 volts for 15 - 45
min. Ethidium bromide was incorporated into the gel and
the running buffer by adding 5 µl ethidium bromide to 500
20 ml running buffer. Running buffer was:

        Tris, 121.16 g
        boric acid, 61.86 g
        EDTA, 7.46 g
25   up to 4 l with water, pH brought to 8.3

Gels were photographed by Polaroid camera at f4.5 for 2
sec using #667 film while being transilluminated by UV
light.
30

Nick translation to label the inter-Alu PCR product with
biotin for use as probe

        Reaction components were from BRL nick translation
35 kit, Cat. No. 8160SB. (BRL, Gaithersberg, MD).

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```
       xµl PCR product (1ug DNA)
       5 ul A1
       2.5 µl 0.4mM Bio-7-dATP
       y µl H₂O
 5     45 µl
add 5 µl solution C (DNA pol.1 and DNase)
       50 µl
```

Product was mixed, spun down, and then incubated at
10   16°C for 90 min. 5 µl stop buffer was added and the
mixture then run through a Worthington Sephadex column to
remove free nucleotides. A check for DNA was made using
100 ng on minigel. The size of inter-Alu PCR product
ranged from 0.1->4kb and the size of the nick translation
15   product ranged from 100-400 bp.

Slide Preparation

1.   Freshly fixed (acetic acid-methanol) slides of cells
20   in log phase growth were prepared by standard methods.

2.   RNAse treatment. 100 ug/ml, 1 hr., moist chamber,
37°C, rinse in 4-6 changes in 2xSSC, dehydrate through
ethanols.
25

3.   Denature DNA. Immerse in 70% formamide (in 2x SSC)
at 71°C for 5 min. Dehydrate in ethanol on ice. Dry.

Probe Cocktail for *In-Situ* Hybridization -- Preparation and Application

In an Eppendorf tube was added 400 ng biotinylated
probe DNA, 20 ug of Cot 10 human DNA (see next paragraph)
and 5 ug of sonicated salmon sperm DNA. The DNA was
dried down, and resuspended in 5 μl H_2O before adding 12.5
μl formamide, 2.5 μl 10xSSC, and 5 μl 50% dextran
sulphate. Probe was denatured at 75°C for 5 minutes,
pre-anneal at 37°C for 20 minutes. The probe was applied
on one slide, covered with a 22 x 30mm coverslip and
Incubated at 37°C overnight in moist chamber.

Washes were conducted at 41°C: 3 changes in 50%
formamide in 2 x SSC for 3 min. each, then 5 changes in 2
x SSC for 2 min each stirring vigorously. Storage was in
the dark in BT buffer, pH 8.0.

Cot 10 human DNA was prepared from placental DNA,
purchased from Sigma. It was dissolved in TE and
sonicated to an average size of 500 bp. Then 20 mg of
DNA was denatured and incubated at 65°C in 2 ml 5 x SSC
until Cot10. The DNA solution was quickly chilled on ice
and 10 fold excess of nuclease S1 buffer (33 mM NaAc,
pH4.5 and 1 mM $ZnSO_4$) and 800 units of nuclease S1 were
added. After 2 hours incubation was carried out at 37°C
to digest single-stranded DNA before removing nuclease S1
with proteinase K digestion. Finally the DNA was
purified with phenol/chloroform extraction and ethanol
precipitation.

Fluorescent Staining

The following steps were utilized in fluorescent
staining procedures.

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1.   Drain and blot off BT buffer and add 55 µl 5% BSA in
BT buffer and cover with plastic cover slip 5 min at room
temp.

5   2.   Peel off cover slip, drain fluid and put on 55 µl
fluorescein avidin solution (2.5 µl fluorescein avidin
(FA) and 247.5 µl 5% BSA in BT buffer). (That comes to
0.55 µl FA.) Cover with plastic cover slip, in moist
chamber 37°C for 1 hr.

10

3.   Wash in BT buffer at 40°C 3x. Store as before in
dark in BT buffer at room temp.

4.   Blot and apply 55 µl 5% goat serum in BT buffer and
15   cover with plastic cover slips for 5 min. at room temp.
Peel off cover slip, drain fluid and add 55 µl of
biotinylated anti-avidin solution (12.5 µl biotinylated
anti-avidin plus 237.5 µl 5% goat serum in BT buffer and
recover with plastic cover slip). Incubate in moist
20   chamber at 37°C for 1 hr. and wash as in "3".

5.   Add another layer of fluorescein avidin as in "2"
above.

25   6.   Repeat "3", "4", and "5" to put on a 3rd layer.

7.   Wash and store as in "3".

8.   Counterstain for 90 sec. in 2µl/ml propidium iodide
30   in deionized water. Mount with antifade solution (38).

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Microscopy

Photomicrographs were obtained using a Zeiss Epi-
illumination Photoscope with filter combination 48 77 09
5   and photographed on Kodak Ektachrome 160 with exposure
times between 30 and 50 seconds.

The following examples are intended to illustrate
the practice of the present invention and are not
10   intended to be limiting.

Example 1

15

Selective Amplification of Human DNA with Alu-1 and Alu-2.

The primers were tested for their specificity for
20   amplification of human DNA. At standard conditions for
PCR (which include annealing of primer to template DNA at
55°C) both primer sets Alu-1 and Alu-2, either alone or
in combination, amplified rodent as well as human DNA.
This is demonstrated in Panel A of Figure 1. The
25   products of the PCR reaction conducted on the DNA from
human HeLa cells (human) and CHO cells (Chinese hamster)
were visualized after electrophoretic separation on an
agarose gel. A smear of product (indicating DNA in a
wide range of molecular weights (from 100 bp to 20,000
30   bp) was produced in CHO as well as human DNA. However,
raising the annealing temperature to 65°C successfully
amplified human but not CHO DNA. This is indicated in
Panel B of Figure 1 where a wide size range of DNA was
produced from human DNA but none from CHO.

35

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Example 2

The efficiency of the Alu-1 and Alu-2 primer sets
were compared with Alu-PCR primers TC-65 or 559
5   previously published (19) and used to generate *in-situ*
-hybridization probe (20). This was done by using a
series of cosmids containing 20,000 bp to 40,000 bp of
human DNA as substrate for PCR reactions and separating
the products on agarose gels. The more efficient primers
10   were expected to prime more frequently and therefore
produce a wider spectrum of DNA bands from the cosmids.
As shown in Figure 2, Alu-1 and Alu-2 primers (O)
produced a significantly greater number of bands of human
genomic DNA than were produced using the previously
15   published primer (N) from each cosmid.

Example 3

PCR Production of Human Probe from Hybrid Cells

20

Cell line 5HL9-4 is a human x CHO somatic cell
hybrid containing chromosome 19 as its only human genomic
element (39). This is demonstrated in Figure 3 where
using total human DNA as *in-situ* hybridization probe on
25   the cells revealed only a single human chromosomal
element as brightly fluorescing. Conducting PCR with
Alu-1 and Alu-2 as primers, at the annealing temperature
(65°C) specific for human DNA, produced a smear of human
DNA from 100 bp to 20,000 bp in length. Therefore, using
30   the specific hybrids and conditions for PCR, human
specific probe for *in-situ* hybridization could be
prepared from DNA of a hybrid cell in which the human DNA
represented less than 5% of the total in the cell.

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Example 4

Painting of a Specific Human Chromosome

5   Inter-Alu-PCR DNA from 5HL9-4 was used for *in-situ*
hybridization on a human metaphase cell after
biotinylating the PCR product, blocking non-chromosome
specific repeat sequences with low Cot DNA and
visualizing the sites of hybridization with the avidin-
10   fluorescence reaction. The results, shown in Figure 4,
clearly indicated human lymphocyte metaphases in which
the only brightly fluorescing human genetic elements were
the two human number 19 chromosomes. Significant
fluorescence was present on only the two human number 19
15   chromosomes while fluorescence on other chromosomes was
effectively quenched. Note the absence of fluorescence
at the centromere of this and all other chromosome
specific probe produced by inter-Alu-PCR. At this level
of contraction, each chromosome arm of chromosome 19
20   appears as one large R-band.

Example 5

Painting a Specific Chromosome Arm

25
       The procedure described was used to paint only part
of a human chromosome. Figure 5 indicates a hybrid cell
(41XP91-3) containing a fragment of a human chromosome.
41XP91-3 is one of a series of hybrid cells made between
30   human xeraderma pigmentosum cells and the DNA repair
deficient CHO cell line UV41. Hybrids were selected for
the complementation of the missing DNA repair function in
UV41 (selection in mitomycin C). Biochemical and
molecular analysis of the hybrids indicated that the
35   human repair gene complementing the lost function in UV41
must be on human chromosom 16 sinc genetic markers

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identifying the 16 were present in every hybrid while
other human chromosomes were only randomly present (40).
Furthermore, the analysis indicated that 41XP91-3 had
markers only from the p-arm of chromosome 16 and

5   contained no markers representing any other region of the
genome. That, plus the cytogenetic analysis exemplified
in Figure 5, allowed us to concluded that the hybrid cell
line contained only a portion of human chromosome 16 as
its only human content. Probe was prepared and applied

10   to human metaphases. As seen in Figure 6 only the p-arm
of chromosome 16 lights up.

                          Example 6

15   Painting a Specific Sub-Region or Band of a Chromosome

         Probe was prepared from a hybrid (2F5) containing
only a small fragment of human genetic material attached
to a CHO chromosome (Figure 7). 2F5 was derived from a

20   human x CHO hybrid (20XP3542-1-4) which was shown to have
approximately 25 Mb of human DNA derived largely from a
portion of human chromosomes 17 (41) and a small region
from the q-arm (q13.3) of chromosome 19 in the vicinity
of the DNA repair gene ERCC1 (42). 2F5 was prepared from

25   20XP3542-1-4 by X-irradiation of the former and
hybridization back to the CHO cell line that was its
original rodent parent (UV20). Radiation hybrids were
selected for ERCC1 by growth in mitomycin C (43).
Analysis of molecular markers retained in 2F5 indicated

30   that it had lost all human DNA except the region of ERCC1
on chromosome 19 and that the total amount of human DNA
in the hybrid was between 1 Mb and 2 Mb (D. Shaw,
Cardiff, Wales, U.K., personal communication). The in-
situ hybridization probe made by inter-Alu-PCR from 2F5

35   detected only band p13.3 on every human chromosome 17 in
all human metaphases seen (over 50; e.g., Figure 8).

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Example 7

Painting Specific Chromosomes With a Banding Pattern

5   Probe was prepared from a hybrid containing only a
single human genetic element which molecular analysis
indicated to be a human chromosome number 7. When probe
was made from this hybrid and used for *in-situ*
hybridization on human metaphases (Figures 9 and 10), the
10   two chromosome 7s lit up in an R-banding pattern.

         Probe was made from the DNA of a hybrid known to
contain only chromosome 5 (obtained from L. Nagaragian,
Department of Hematology, University of Texas M.D.
15   Anderson Cancer Center, Houston, TX). As shown in figure
11, chromosome 5 is the only painted element in a human
metaphase spread. The distinctive R-band pattern is
identifiable in this somewhat overly contracted state.

20   Inter-Alu-PCR probe made from a hybrid containing
only human chromosome 17 (obtained from David Ledbetter,
Baylor College of Medicine, Houston, TX) specifically lit
up chromosome 17 in human metaphase spreads (Figure 12).
The chromosome is small and highly contracted in this
25   metaphase. As seen in Figure 13, the R-band patterns are
resolved and the chromosome 17s are observed in
mataphases where chromosomes are not well spread out.

         Probe made from a hybrid containing human chromosome
30   3 as the only human element was characterized and
obtained from Dr. Susan Naylor (University of Texas
Health Science Center at San Antonio, TX). When applied
to a human metaphase spread this probe specifically
hybridized to human chromosome 3 in an R-banded pattern
35   (Figure 14) supporting the hypothesis constructed after
observing the results with th chr m some 7 probe.

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In addition to the human chromosomes presented in
the examples, the method has been used to paint R-band
patterns on chromosomes 9 and 16.

5   Example 8

Detection of Chromosomal Abnormalities

From the examples shown, it is clear that
10   translocations between one chromosome and another will be
obvious and easily detected using a chromosome specific
probe. For instance, where a translocation existed
between one of the chromosome 19s and one of the 17s,
probe from the 19 would readily detect a single, entirely
15   fluorescing chromosome (the 19 not involved in the
translocation) and two other chromosomes with only
portions fluorescing (the 19 with a piece of the 17 and
the 17 with a piece of the 19). By the same token, it
will be even easier to identify monosomies (a single
20   fluorescing chromosome instead of two in the cell), and
trisomies (three fluorescing chromosomes). A special
advantage in working with probes for only a portion of a
human chromosome or which light up a banding pattern on a
chromosome is that it will enable the identification of
25   inversions and also the specific sites of deletions and
translocations.

Detection of an Inversion

30   A chromosome 16 p-arm specific probe as described in
Example 4 above was used to identify the inversion 16
chromosome associated with acute nonlymphocytic leukemia.
Since the inversion breakpoint on the p-arm cuts right
through the painted region of the chromosome, the
35   inversion chromosome was readily identified as a
chrom some having flu rescence at the end f the p-arm

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and also in the middle of the q-arm (Figure 15).

Detection of the Site on a Deletion

5   Chromosome 7 specific probe which lit up a banding
pattern on chromosome 7 was used to identify the location
of a deletion on the 7 associated with an AML cell line
KB-1 (provided by Jan Liang, Division of Laboratory
Medicine, University of Texas M.D. Anderson Cancer
10   Center, Houston, TX). When applied to the cell, the
number 7 chromosomes were immediately identifiable with
the normal 7 quite distinct from the deleted 7. The site
of the deletion was readily detected as just distal to
the most proximal bright band on the q-arm as shown in
15   Figure 16.

Example 9

Identification of Chromosome Regions in Interphase Nuclei
20

Chromosome Regions in Normal Cell Interphases

In the interphase nuclei, chromosomes are "unwound"
or drawn out to the extent that they are no longer
25   individually identifiable. Using the probes that are
specific for an entire human chromosome, it is possible
to identify but difficult to resolve the "domains" within
an interphase nucleus in which the particular chromosomes
exist. However, by using probe made from a hybrid
30   containing a highly limited region of a particular
chromosome, such domains are clear, bright and highly
resolved. This is demonstrated by using probe made from
hybrid 2F5 (described in Example 6 above) which contains
only 1 - 2 Mb of human DNA from band q13.3 of human
35   chromos me 19. As seen in Figure 20, such probe gives
clear res lution f the chr mosomal region in interphase

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nuclei.

Chromosomal Abnormalities in Interphases

5   Probe for any normal chromosome will identify two
domains in an interphase nucleus. Where there has been a
translocation involving a chromosome three, poorly
resolved domains may be visible. Three domains were
expected from the inversion 16 chromosome described in
10   Example 8a above. Figure 19 shows that by using probe
for only part of the chromosome in such an interphase
nucleus, the normal 16 appeared as a single elongated
domain of fluorescence representing the uninvolved 16p
arm. Also seen was a second elongated domain divided by
15   a zone of non-fluorescence as expected for the chromosome
bearing the inversion.

PROPHETIC EXAMPLE 10

20   The present example outlines the procedure
contemplated by the Applicants to be useful for the
isolation of genes that have been localized to a known
region of the genome.

25   Probe is made from a specific region of the human
genome where the gene of interest is known to be
localized (via classical genetic mapping studies in
families where that gene is known to be segregating, for
example). A gene demonstrated to map into the band q
30   13.3 of chromosome 19, for example, is screened from cDNA
libraries made from genes expressed in the human brain
using inter-Alu-PCR probe made from the hybhrid 2F5
(which retains band 19q13.3 as its only human genetic
material). This will result in isolation of human brain
35   genes fr m the genom region in which the desired g ne is
known to exist, providing candidate gen s for the gene of

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interest. Isolation of such a gene would be viewed as
important in the characterization, diagnosis, counselling
and treatment of any disease associated with that gene.

5   Figure 20 is a schematic illustration of the
invention. In order to illustrate a particular example
of how the primer sets work, a human Alu repeat sequence
is shown in Figure 20A. Figure 20B illustrates how
nonrepeat DNA regions are selectively amplified.
10   Although the figure is illustrated using human Alu repeat
segment, the same general scheme applies to the
preparation of chromosomal specific DNA probes using
other species specific repeat sequences.

15   The present invention has been described in terms of
particular embodiments found by the inventors to comprise
preferred modes of practice of the invention. It will be
appreciated by those of skill in the art that in light of
the present disclosure numerous modifications and changes
20   can be made in the particular embodiments exemplified
without departing from the intended scope of the
invention. For example, primers and primer sets to
repeat sequences other than Alu could be designed using
the principles set forth without affecting the intended
25   scope of the invention. All such modifications are
intended to be included within the scope of the claims.

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by reference to the extent they supplement, explain,
provide a background for or teach methodology, techniques
and/or compositions employed herein.

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CLAIMS:

1.   A method for the production of chromosome specific
DNA probes essentially free of species specific repeat
5   DNA sequences, comprising the steps:

identifying a desired chromosome or portion thereof
from a species to which chromosome specific
probes are desired;

10

obtaining DNA from a source in which the desired
chromosome or portion thereof has been isolated
from the species total chromosomal material;

15   preparing a first and a second primer set, each
primer set capable of hybridizing to a region
within a species specific repeat DNA segment
wherein the first primer set binds at or near
the repeat segment 5' terminus of the
20   chromosome DNA and facilitates DNA replication
in the direction of that terminus and the
second primer set binds at or near the repeat
segment 3' terminus of the chromosome DNA and
facilitates DNA replication in the direction of
25   that terminus;

annealing the primer sets with the DNA obtained from
the source in which the desired chromosome or
portion thereof has been isolated from the
30   species total chromosomal material; and

amplifying the DNA from inter-repeat sequence
regions of the desired chromosome or portion
thereof to produce DNA probes essentially free
35   of species specific repeat sequences.

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2.   The method of claim 1 wherein each primer set
comprises at least one oligonucleotide.

5   3.   The method of claim 1 wherein the chromosome or
portion thereof identified is characterized as having DNA
specific for the chromosome or portion of the chromosome.

10   4.   The method of claim 1 wherein the DNA probes are
essentially free of alphoid, LINE, or SINE repeat
sequences.

15   5.   The method of claim 4 wherein the SINE is Alu.

6.   The method of claim 1 wherein the chromosome or
portion thereof identified is characterized as having
20   species specific Alu segments.

7.   The method of claim 1 wherein the amplifying is by
polymerase chain reaction.
25

8.   The method of claim 1 wherein the chromosome or
portion thereof is human.

30
9.   The method of claim 8 wherein the chromosome portion
is a chromosome segment isolated in an interspecific cell
hybrid or a yeast artificial chromosome.

35
10.   The method of claim 1 wherein the first primer set

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binds to at least a portion of a species specific repeat
DNA consensus sequence at or near the repeat sequence 5'
terminus.

5

11.   The method of claim 1 wherein the first primer set
binds to a species specific repeat DNA consensus sequence
located at or within 50 base pairs of the repeat sequence
5' terminus.

10

12.   The method of claim 1 wherein primers comprising the
first primer set are characterized as having a reverse
complementary sequence to a consensus sequence located
15   within a repeat segment.

13.   The method of claim 12 wherein the repeat segment is
a human Alu segment.

20

14.   The method of claim 12 wherein the consensus
sequence is located 13-31 base pairs from a human Alu 5'
terminus.

25

15.   The method of claim 1 wherein primers comprising the
second primer set bind to a species specific repeat
consensus sequence located at or near the 3' terminus.

30

16.   The method of claim 1 wherein primers comprising the
second primer set bind to a species specific repeat
sequence located at or within 100 base pairs of the
35   repeat sequence 3' terminus.

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17. The method of claim 1 wherein primers comprising the second primer set bind with a human Alu consensus segment located 240-258 base pairs from an Alu 3' terminus.

5

18. The method of claim 17 wherein primers comprising the second primer set are characterized as having a sequence identical to the human Alu repeat sequence located from base pair 240-258.

10

19. The method of claim 1 wherein each first and second primer set is characterized as comprising at least one primer, said primer or primers binding with a plurality of species specific repeat segments.

15

20. The method of claim 19 wherein the primer or primers comprising the first and second primer sets are further characterized as binding essentially only to human Alu segments.

20

21. The method of claim 1 wherein obtaining DNA of the desired chromosome isolated from the species total chromosomal material is by flow sorting.

25

22. The method of claim 1 wherein obtaining DNA of the desired chromosome or portion thereof isolated from the species total chromosomal material is by microdissection.

30

23. The method f claim 1 wherein the sourc f the desired desired chromosome or portion th reof is interspecific somatic cell hybrids.

35

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24. The method of claim 1 wherein DNA obtained from interspecific somatic cell hybrids is annealed to primers at a temperature between about 60°C and 80°C.

5

25. The method of claim 23 wherein the annealing temperature is 65°C.

10   26. The method of claim 23 wherein the interspecific somatic cell hybrid is hamster x human.

27. The method of claim 1 wherein the source of the
15   desired chromosome portion is artificial yeast chromosomes.

28. A method of producing DNA probes specific for a
20   human chromosome or portion thereof, comprising the steps:

        obtaining DNA from a source in which the desired
            human chromosome or portion thereof has been
25   isolated from other human chromosomal material;

        preparing a first and second primer set, each primer
            set capable of hybridizing to a region within a
            human specific repeat DNA segment wherein the
30   first primer set binds at or near the repeat
            segment 5' terminus of the desired human
            chromosome DNA and facilitates DNA replication
            in the direction of that terminus and the
            second primer set binds at or near the repeat
35   segment 3' terminus f the desired human
            chromosome DNA and facilitates DNA replication

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in the direction of that terminus;

annealing the primer sets with the DNA obtained from
the source in which the desired chromosome or
portion thereof has been isolated from the
human total chromosomal material; and

amplifying the DNA from inter-repeat sequence
regions of the desired chromosome or portion
thereof to produce DNA probes essentially free
of human specific repeat sequences.

29. The method of claim 28 wherein each primer set
comprises at least one oligonucleotide.

30. The method of claim 28 wherein the first set of
primers binds to a consensus sequence within a human Alu
repeat segment.

31. The method of claim 30 wherein the consensus
sequence is located within 50 base pairs of the human Alu
repeat segment 5' terminus.

32. The method of claim 30 wherein the first set of
primers binds to a sequence located between base pairs
13-31 of a 5' end of a human Alu repeat consensus
segment.

33. The method of claim 28 wherein the primers
comprising the first set of primers differ in one or more
base positions whereby said primers bind to essentially

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all sequences located between base pairs 13-31 of human
Alu repeat segments.

5   34.   The method of claim 33 wherein said primers
comprising the following base sequence:

     GGATTACAGG YRTGAGCCA

10   where Y is T or C and R is A or G producing four primers
in the set.

     35.   The method of claim 33 wherein the first set of
15   primers comprises approximately equal amounts of four
primers.

     36.   The method of claim 28 wherein the second set of
20   primers binds to a consensus sequence within a human Alu
repeat segment.

     37.   The method of claim 36 wherein the consensus
25   sequence is located within 100 base pairs of the human
Alu repeat segment 3' terminus.

     38.   The method of claim 28 wherein the primers
30   comprising the second set of primers differ in one or
more base positions whereby said primers bind to
essentially all sequences located between base pairs 240-
258 of human Alu repeat segments.

35

     39.   The method of claim 38 wherein said primers have the

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following base sequence:

　　　　RCCAYTGCAC TCCAGCCTG

5　　where Y is T or C and R is A or G producing four primers
　　in the set.

10　　40. The method of claim 38 wherein the second set of
　　primers comprises equal amount of four primers.

　　41. A method for painting a human chromosome or portion
　　thereof in metaphase spreads or interphase nuclei,
15　　comprising the steps:

　　　　preparing human cell sample in which a particular
　　　　　　human chromosome or portion thereof is to be
　　　　　　detected;

20
　　　　preparing a DNA probe by the method of claim 28;

　　　　labeling the DNA probe;

25
　　　　hybridizing the labeled DNA probe with the cell
　　　　　　sample;

　　　　detecting the human chromosome or portion thereof in
30　　　　　　metaphase spreads or interphase nuclei
　　　　　　hybridized with the DNA probe.

　　42. The method of claim 41 further comprising blocking
35　　or removing non-chromosome specific r peat sequences that
　　may be present in the DNA probe.

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43. The method of claim 41 wherein R-banding patterns
are painted onto specific human chromosomes in metaphase
spread.

5

44. The method of claim 41 wherein a human chromosome or
portion thereof comprises at least 500 chromosome
specific DNA base pairs.

10

45. The method of claim 41 wherein the particular human
chromosome or portion thereof detected is 100-2000 kb.

15   46. The method of claim 41 wherein the labeling is with
biotin, digoxigenin, dinitrophenol, aminoacetylfluorene,
or conjugated mercury.

20   47. The method of claim 41 wherein the detecting is by
fluorescence microscopy following conjugation with an
antigenic fluorochrome.

25   48. The method of claim 47 wherein the antigenic
fluorochrome is avidin-fluorescein.

49. The method of claim 42 wherein the blocking
30   comprises binding non chromosome specific regions of the
DNA probe with middle and highly repetitive DNA isolated
from total human DNA.

35   50. The method of claim 49 wherein the middle and highly
repetitive DNA has a C_0t value of 10.

51. The method of claim 42 wherein the removing comprises the following steps:

    obtaining a plasmid containing a complete human Alu
5   repeat segment;

    preparing a first plasmid arm primer from a known
        sequence of the plasmid arm adjacent to the 5'
        terminus of the Alu insert, wherein the primer
10   will direct DNA synthesis toward an Alu-1
        primer, said Alu-1 primer having a base
        sequence reverse complementary to a consensus
        sequence near the 5' end of the Alu repeat
        segment and directing DNA synthesis toward the
15   plasmid arm primer;

    preparing a second plasmid arm primer from a known
        sequence of the plasmid arm adjacent to the 3'
        terminus of the Alu insert, wherein the primer
20   will direct the DNA synthesis toward an Alu-2
        primer, said Alu-2 primer having a base
        sequence identical to a consensus sequence near
        the 3' end of the Alu repeat segment and
        directing DNA synthesis toward the plasmid arm
25   primer;

    polymerase chain reaction amplifying both Alu
        terminal regions located between each plasmid
        arm and the Alu consensus sequences binding to
30   Alu-1 or Alu-2 primer;

    adding human C_0t 10 DNA;

    linking the amplified Alu terminal regions and human
35   C_0t 10 DNA to a carrier;

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       hybridizing the carrier-linked Alu terminal regions
and C_0t 10 DNA with the labeled probe; and

       separating carrier with bound non-chromosomal
5   specific repeat sequences from labeled probe.

52. The method of claim 51 wherein the plasmid is BLUR2
plasmid DNA.
10

53. The method of claim 51 wherein the carrier is
diazotized Sephacroyl.
15

54. The method of claim 51 wherein the carrier is
diazobenzoxymethyl cellulose covalently linked to non-
chromosomal specific repeat DNA sequences.
20

55. The method of claim 1, 28 or 41 wherein the portion
of chromosome is chromosome 16 p-arm, 19 q13.3, 1q, 3q,
5q, 7q, 9q or 22 q.
25

56. The method of claim 1, claim 28 or claim 41 wherein
the desired chromosome is human chromosome 1-22, x-
chromosome or y chromosome.
30

57. A kit useful for preparing probes for inter-Alu-PCR
specific for human chromosomes or parts of human
chr m somes comprising:
35

       a transporter being compartmentalized to receive one

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or more container means in close confinement
therein;

a first container means comprising a set of Alu-1
5   primers, said primers having the base sequence
          GGATTACAGG YRTGAGCCA wherein Y is T or C and R
          is A or G; and

a second container means comprising a set of Alu-2
10   primers, said primers having a base sequence
          RCCAYTGCAC TCCAGCCTG wherein Y is T or C and R
          is A or G.

15   58. The kit of claim 57 wherein the primer sets are
     supplied in lyophilized form.

     59. A kit useful for the *in-situ* painting and banding of
20   human chromosomes or parts of human chromosomes
     comprising:

a transporter being compartmentalized to receive one
          or more container means in close confinement
25   therein; and

a first container means comprising labeled, DNA
          probes essentially free of species specific
          repeat sequences capable of binding and
30   painting specific human chromosomes or parts of
          human chromosomes.

     60. The kit of claim 59 wherein DNA probes are labeled
35   with bi tin.

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61.   The kit of claim 59 wherein the DNA probes are supplied in lyophilized form.

5   62.   The kit of claim 59 wherein the probes are specific for human chromosomes 1-22, the X and the Y chromosomes.

63.   The kit of claim 59 wherein the probes are specific
10   for chromosome parts: 16p, 19 q13.3, 9q, 22q, 5q, 7q, 3p, 1q.

64.   A nucleotide primer set comprising the following
15   base sequence:

GGATTACAGG YRTGAGCCA

wherein Y is T or C and R is A or G and wherein the
20   primers are characterized as binding near a 5' Alu terminus in a plurality of human Alu consensus segments.

65.   A nucleotide primer set comprising the following
25   base sequence:

RCCAYTGCAC TCCAGCCTG

wherein Y is T or C and R is A or G and wherein the
30   primers are characterized as binding near a 3' Alu terminus in a plurality of human Alu consensus segments.

66.   A collection of DNA probes capable of hybridizing to a selected region of a chromosome, said probes
35   characterized as essentially fr e of species specific repeat segm nts.

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67. The DNA probes of claim 66 characterized as specific
for human chromosomes 1-22, X chromosome, Y chromosome,
16p, 19q13.3, 9q, 22q, 5q, 7q, 3q and 1q.

5   68. A method of isolating genes identified as associated
with a condition or disease state, comprising preparing a
DNA probe of by the method of claim   and identifying
gene candidates from a cDNA library prepared from a
sample suspected of having the gene associated with the
10   condition or disease state.

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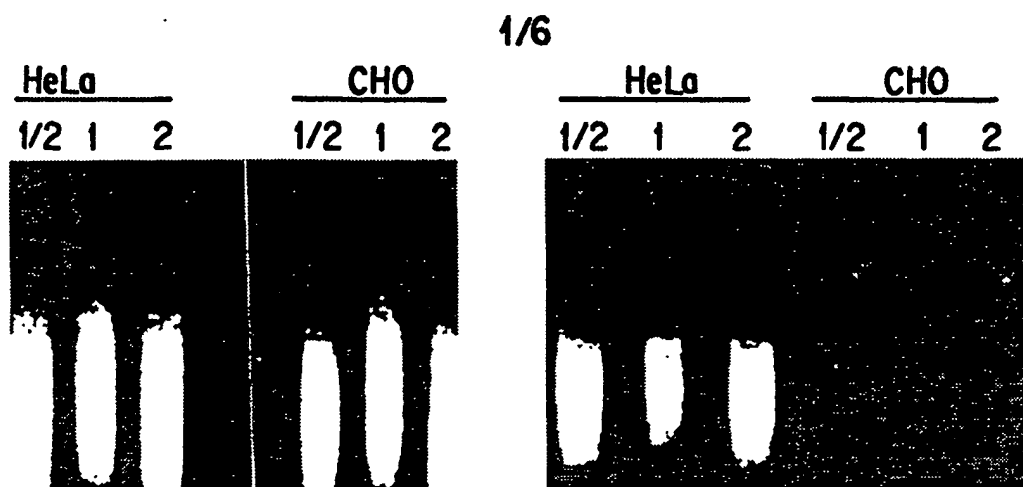
FIG. 1A   FIG. 1B
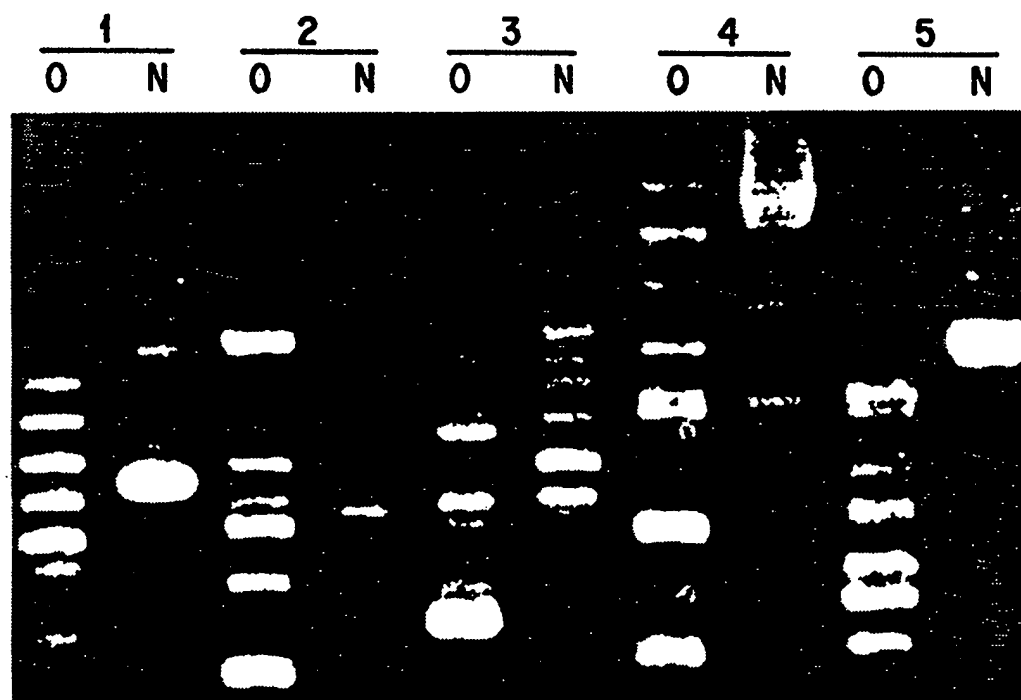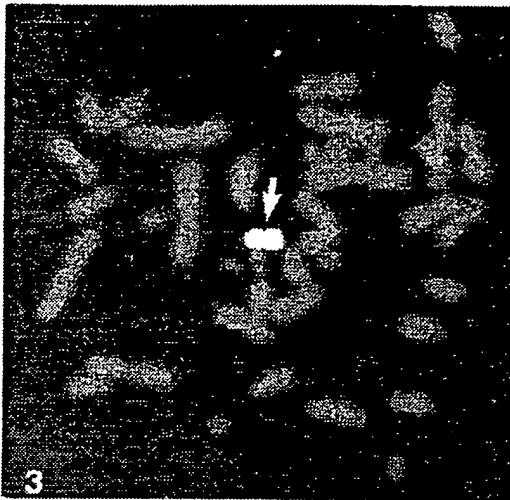
FIG. 2

Fig. 3
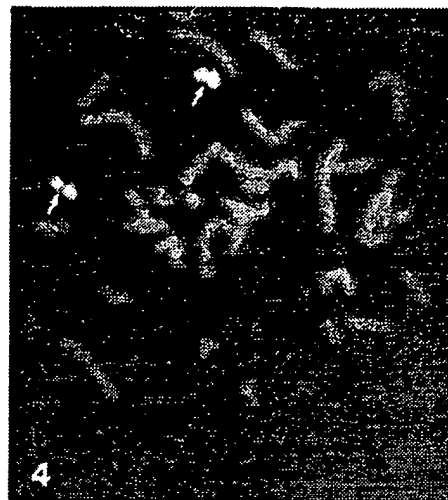
Fig. 4

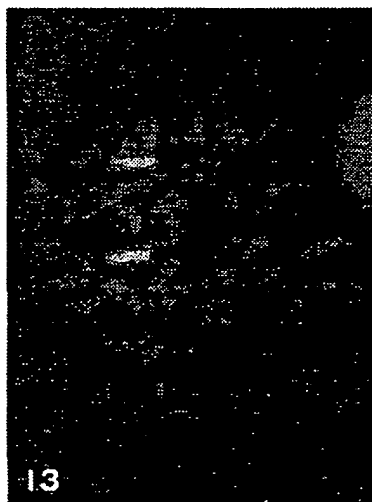
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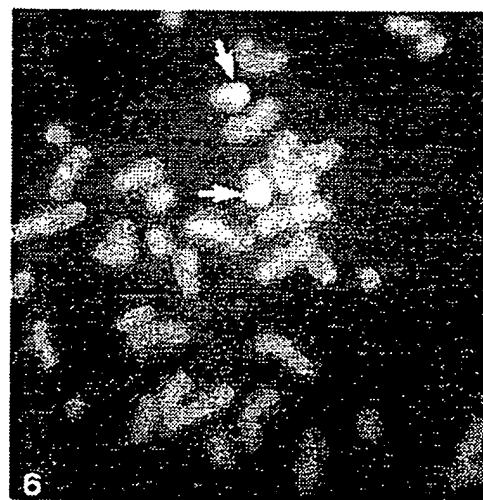
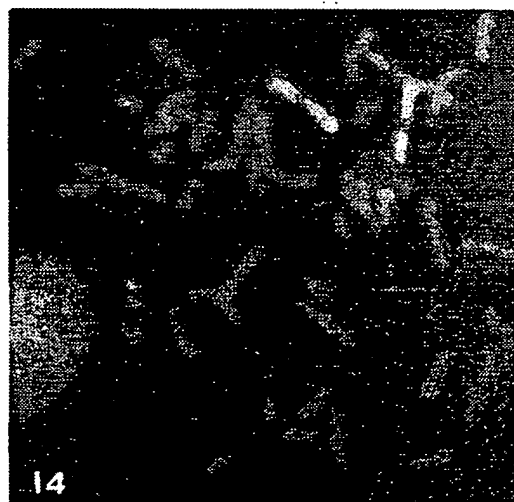Fig. 6
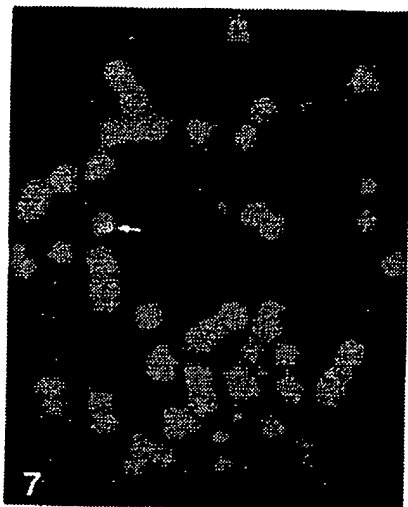
Fig. 7

Fig. 8
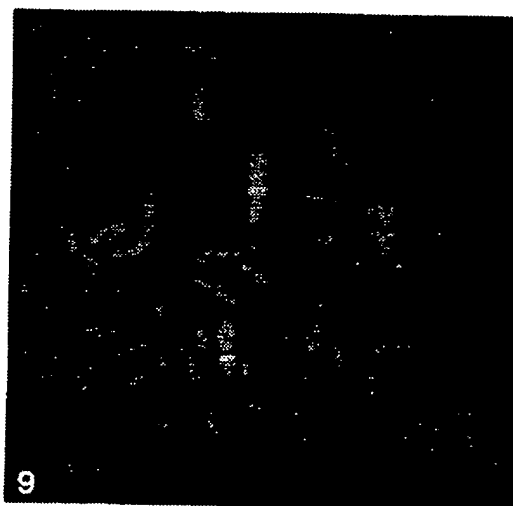
Fig. 9
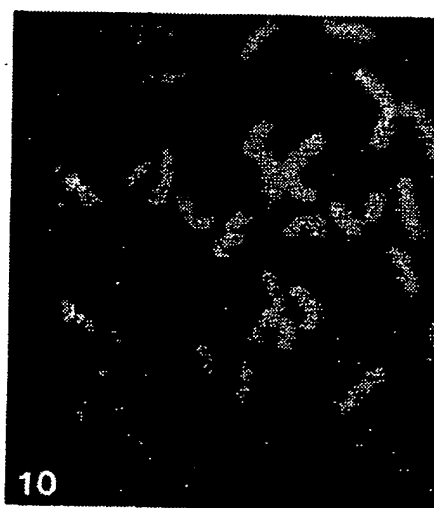
Fig. 10
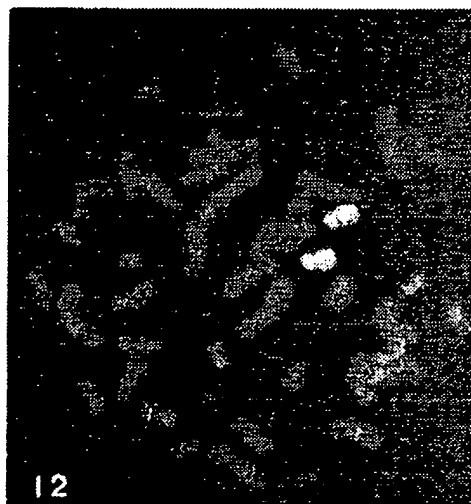
Fig. 11

Fig. 12

Fig. 13

Fig. 14
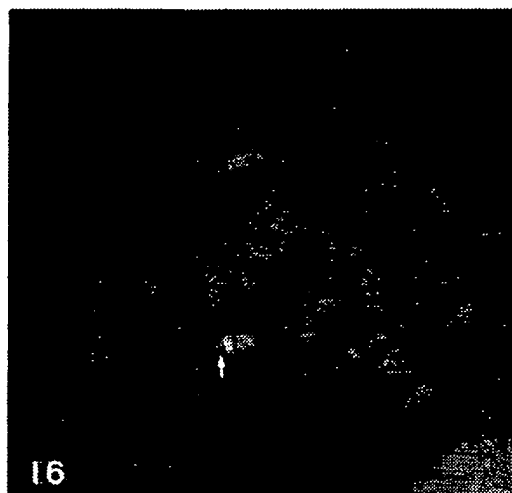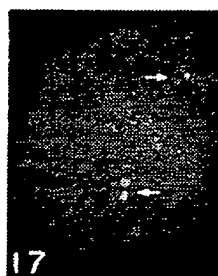
Fig. 15

Fig. 16

Fig. 17

Fig. 19
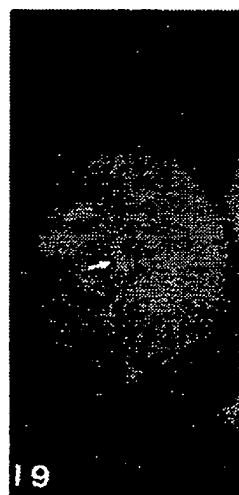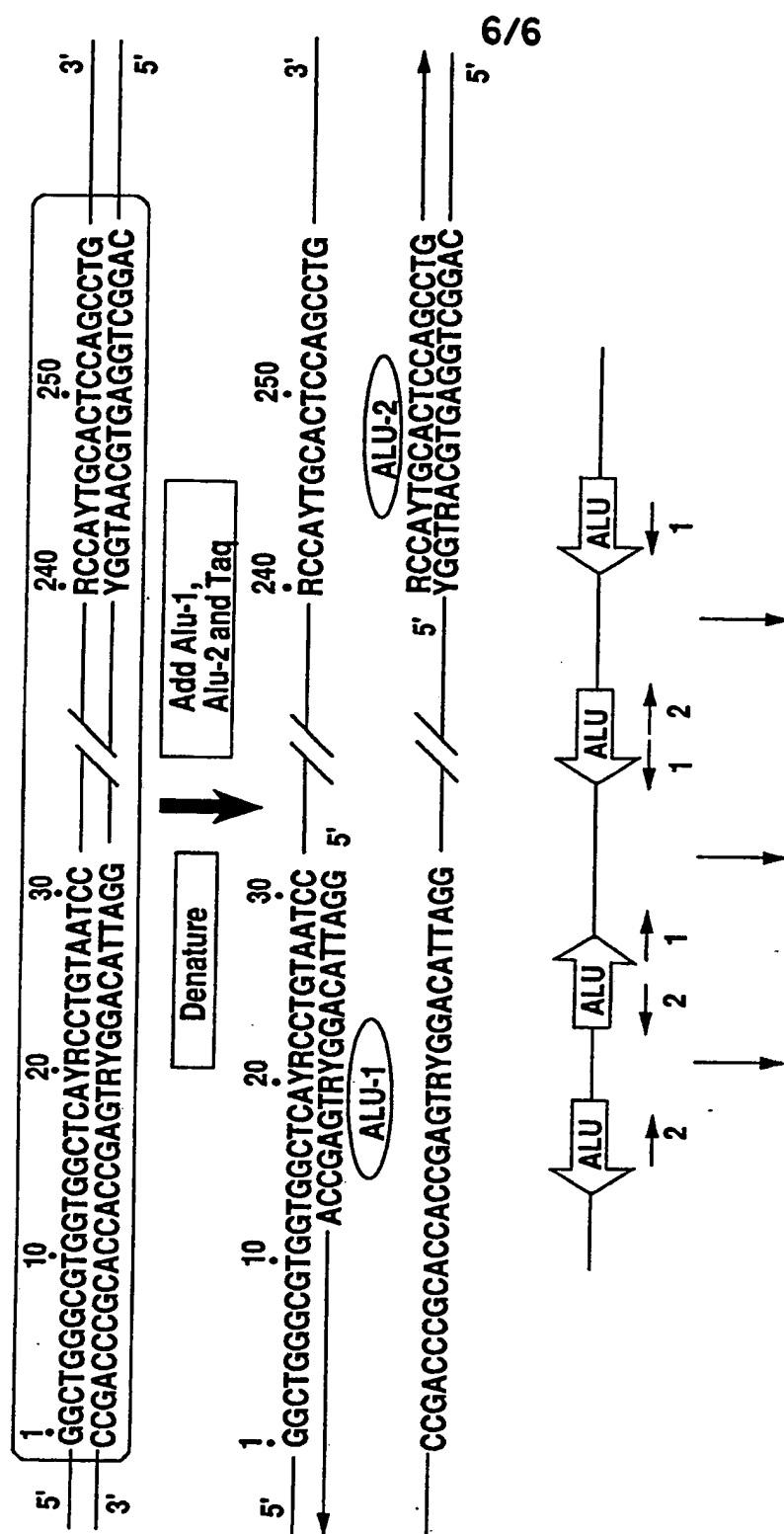
Fig. 18

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5' GGCTGGGGCGTGGTGGCTCAYRCCTGTAATCC　　　　RCCAYTGCACTCCAGCCTG 3'
3' CCGACCCCGCACCACCGAGTRYGGACATTAGG　　　　YGGTAACGTGAGGTCGGAC 5'

　1　　　10　　　20　　　30　　　240　　250

↓ Denature

↓ Add Alu-1, Alu-2 and Taq

5' GGCTGGGGCGTGGTGGCTCAYRCCTGTAATCC　　　　RCCAYTGCACTCCAGCCTG 3'
　　　　　　　　　　ACCGAGTRYGGACATTAGG 5'

　1　　　10　　　20　　　30　　ALU-1　　　240　　250

3' CCGACCCCGCACCACCGAGTRYGGACATTAGG　　　　RCCAYTGCACTCCAGCCTG
　　　　　　　　　　　　　　　　　　5' RCCAYTGCACTCCAGCCTG
　　　　　　　　　　　　　　　　　　　YGGTRACGTGAGGTCGGAC 5'
　　　　　　　　　　　　　　　　　　ALU-2

FIG. 20

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/10;   C12Q1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12Q

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	HUMAN GENETICS vol. 86, no. 1, November 1990, BERLIN, DE pages 1 - 6; C. LENGAUER ET AL.: 'Painting of human chromosomes with probes generated from hybrid cell lines by PCR with Alu and L1 primers' see abstract see page 2, left column, line 45 - right column, line 38 --- -/--	1,24,41, 66

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 APRIL 1992	22. 04. 92

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	LUZZATTO E.R.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	GENOMICS vol. 7, no. 2, June 1990, NEW YORK,USA pages 257 - 263; F.E. COTTER ET AL.: 'Rapid isolation of human chromosome-specific DNA probes from a somatic cell hybrid' cited in the application see the whole document esp. abstract, page 258, right column, lines 34 to 47 and " Discussion"	1,28,36, 37
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, September 1989, WASHINGTON US pages 6686 - 6690; D.L. NELSON ET AL.: 'Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources' cited in the application see the whole document esp. abstract and fig. 1	1,56
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 87, September 1990, WASHINGTON US pages 6634 - 6638; P.LICHTER ET AL.: 'Fluorescence in situ hybridization with Alu and L1 polymerase chain reaction probes for rapid characterization of human chromosomes in hybrid cell lines' see the whole document esp. abstract, page 6634, left column, line 23 to right column, line 29 and " Discussion"	1,26,41
A	WO,A,9 005 789 (YALE UNIVERSITY) 31 May 1990 see page 14, line 6 - page 19, line 23; claims	1,41,51
P,X	EP,A,0 430 402 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 5 June 1991 see page 21, line 53 - page 22, line 10; figure 7	1

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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WO-A-9005789	31-05-90	AU-A- CA-A- CN-A- EP-A-	4642089 2003051 1046392 0444115	12-06-90 15-05-90 24-10-90 04-09-91
EP-A-0430402	05-06-91	AU-A- CA-A- JP-A-	5898790 2021489 3224499	06-06-91 20-01-91 03-10-91